Allergen immunotherapy: What can and cannot be mixed?

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Allergen immunotherapy is performed with allergenic products derived from a wide variety of sources in differing formulations, dilutions, and expressed potencies. The use of standardized allergen products is leading to more uniform potency designations with these products, but only a few products have been standardized. Increased information regarding allergen cross-reactivity and the stability of allergens is becoming available, but the scientific knowledge is still incomplete. Despite these problems and the lack of a standardized approach to immunotherapy, a growing body of knowledge is becoming available to move toward uniform formulation practices.

The selection, total number, and proportions of allergen components that are included in therapeutic mixtures are critical aspects of formulating allergen immunotherapy. From a practical perspective, it is tempting to include all relevant allergens into a single vial in equal proportions. This approach can eliminate the need for multiple injections and potentially reduce cost and time. However, this potential gain can be at the expense of clinical efficacy. Based on current evidence for effective doses for allergen immunotherapy, the total number of allergenic extracts that theoretically can be added to a therapeutic mixture is between 7 and 15, depending on the potency of stock allergen extracts used and the volume administered to the patient. The incremental additions of allergenic extracts could reduce the final concentrations of the individual components in the mixture and thus reduce their clinical effect. The preparation of allergen immunotherapy vaccines with an increasing number of extracts creates a potential for interactive effects that can change their allergenic and immunogenic properties. Of particular importance is the contribution of proteolytic enzymes in some fungal and whole-body insect allergenic extracts. For these reasons, guidelines are needed to reduce the number of extracts to include in allergen immunotherapy mixtures.

CROSS-REACTIVITY OF ALLERGENS

An increasing amount of information now exists in the literature that allows for the selection of only a limited number of representative allergens from cross-allergenic groups in allergy diagnosis and immunotherapy.[1,2] The application of this information could greatly simplify formulation practices and reduce inventory requirements. The use of a single dominant and representative pollen species or a stock mix of cross-reacting species should be considered to achieve this goal. For example, 3 grass pollen extracts representing the cross-reacting grass pollen groups (eg, Pooideae [meadow fescue, timothy, orchard, perennial rye, Kentucky blue, and red top grasses], Chloridoideae [Bermuda grass], and Panicoideae [Johnson grass]) can be used to account for essentially all of the grass allergen specificities in the United States. Cross-reactive allergens within taxonomic groups (eg, Chenopodiaceae [scales, lamb's quarter, Russian thistle, and Kochia], Amaranthaceae [pigweeds and Western water hemp], Cupressaceae [juniper, cedar, and cypress], Betulaceae [birch, alder, hazel], Fagaceae [beech and oaks], Oleaceae [ash, olive, and privet], or Salicaceae [cottonwood, poplars, aspen, and willows]) have been identified and should serve to further consolidate allergen choices. Additionally, the grouping of cross-reacting allergen extracts/vaccines to establish target maintenance doses might prove effective in defining the proportions of allergens included in therapeutic mixtures. A review of named-patient prescriptions requested by US allergists and processed at Greer Laboratories indicated that the number of allergen extracts included in nearly 25% of the allergen immunotherapy mixtures could be reduced by nearly one half if information about cross-reactivity was taken into consideration.[3]

Cross-reactivity among unrelated allergen sources can also occur in some patients because of shared IgE-binding epitopes on homologous allergenic molecules, such as the calcium-binding proteins and profilins that have been identified in most allergenic pollen species. For example, patient reactivity to these "pan-allergens" could unnecessarily lead to the inclusion of multiple allergens in the patient's therapeutic mixture. Diagnostic tests with the precision required to identify patients who belong to this group are not yet commonly available. However, progress with such molecular-based diagnostic approaches[4] is being made, and efforts to include them in formulating allergen immunotherapy should be encouraged.

COMPATIBILITY AND STABILITY OF ALLERGEN MIXTURES

Each manufacturer, by law, must have an ongoing stability testing program as part of their product licenses for standardized allergenic extracts, but this requirement is applicable only to supporting the shelf-life of individual allergen products and is not required for mixtures. Some studies have examined the stability of allergens in defined mixtures representing common immunotherapy vaccines. Allergenic extracts containing high concentrations of proteolytic enzymes have been implicated in reducing the potency of allergen mixtures and, by inference, their clinical utility.[5] These include preparations derived from some insects and fungi. The susceptibility of allergens to the deleterious effect of fungal and insect proteases varies, with grass pollen allergens being among the most susceptible. For example, the allergenicity of

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Allergenic Extract	Protease-containing Extracts: Insects	Fungi	Mites	Comments
Insects	∅	⊕	⊕	Whole-body insect extracts contain very high protease levels; susceptible to endogenous proteases unless stored in 50% glycerin
Fungi	⊕	⊕	⊕	Fungal extracts do not appear to be adversely affected by proteases;
Mites	∅	∅	⊕	Mite allergens resistant to insect and fungal proteases if stored in ≥ 10% glycerin.
Pollens	⊗	⊗	⊕	Pollen extracts susceptible to insect and fungal proteases; compatible with mite extracts when stored in ≥ 10% glycerin.
Cat hair/epithelia	⊕	⊕	⊕	Fel d 1 in cat extract is highly resistant to fungal and insect proteases
Dog hair/epithelia	⊕	∅	⊕	Dog allergens susceptible to most fungal extracts, but more stable when mixed with insect extracts.

FIG 1. Combinations producing low *(⊗)*, moderate or risky *(∅)*, and favorable *(⊕)* compatibilities when allergenic extracts are mixed with protease-containing insect, fungal, and mite extracts are shown.

grass pollen extract is reduced to less than 15% of the starting potency when mixed with *Penicillium*, *Aspergillus*, *Alternaria*, or *Cladosporium* species extracts after 6 months of storage, even in the presence of 10% glycerin added as a stabilizer. In contrast, several fungal extracts (ie, *Aureobasidium*, *Fusarium*, and *Mucor* species) induced very little change in grass pollen allergen reactivities when stored under the same conditions. In some cases, increasing the glycerin concentration to 50% significantly increased the stability of the mixture and its shelf-life, but the results are not consistent. Thus it is recommended that fungal and insect allergen extracts containing high levels of protease be separated from pollen allergen extracts. The protease content of fungal extracts will vary depending on a variety of factors, including the strain, growth conditions, culture fractions (mycelia, culture medium, or both) harvested, and manufacturing procedures used. These factors will vary from manufacturer to manufacturer, and for this reason, it might be prudent to separate all fungal and insect allergen extracts from pollen allergen extracts unless this information is available.

It is possible that the results of compatibility studies could depend on the sources of allergens because comparisons of analogous products from multiple manufacturers are not generally reported. To address this, the compatibility of grass pollen allergens with dust mite (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*) extracts was examined by using standardized mite extracts from multiple US manufacturers. No detectable loss of allergen reactivity was observed after mixing grass pollen with the various manufacturers' mite extracts at concentrations equivalent to current immunotherapy practice parameter recommendations. This result was consistent with the relatively low protease content of US standardized dust mite extracts derived from 99% pure mite bodies when compared with some European extracts that are derived from spent cultures. In addition, a study was recently initiated by a subcommittee of the American Academy of Allergy, Asthma & Immunology Immunotherapy and Allergy Diagnostics Committee investigating the stability of extract formulations containing standardized dust mite, cat hair, short ragweed pollen, and timothy grass pollen allergenic extracts.[6] When mixed in proportions consistent with target maintenance concentrations recommended in the immunotherapy practice parameters, the potencies were retained for up to 1 year when stored at 2°C to 8°C. At higher dilutions, such as 1:100 vol/vol, without the presence of stabilizers, such as glycerin or human serum albumin, the potency of timothy grass pollen allergen extract was reduced, but the clinical relevance of this reduction could not be established. Additional testing is expected to be completed this year that could lead to updates in the allergen immunotherapy practice parameters. In the meantime, the compatibility chart (Fig 1) provides some guidelines when formulating mixtures using extracts from insects, fungi, and dust mites, which are known to contain proteases.

The formulation of therapeutic allergen mixtures presents several challenges that require progress in allergen standardization, knowledge of allergen cross-reactivity, allergen compatibility and stability, and the patient's sensitivity and responsiveness to specific allergens. In the past, uniform formulation practices have been difficult to implement, and compliance with the current allergen immunotherapy practice parameter has been difficult to monitor. However, knowledge about allergen cross-reactivity and information about the relative compatibilities and stabilities of individual allergens in mixtures are becoming available, and we should be able to make more informed decisions about what can and cannot be mixed.

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